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Original Article

Radix phytolaccae on tissue metabolomics in rats by gas chromatography-mass spectrometry

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Abstract: Traditional Chinese medicine *Radix phytolaccae* is the dried root of *Phytolacca acinosa Roxb* and *Phytolacca americana L. Radix phytolaccae* has multiple efficacies of antibiosis, antivirus, antiinflammatory, antitumor, enhancing immunity, eliminating phlegm and relieving asthma. In this study, we developed a tissue (liver, kidney and heart) metabolomic method by gas chromatography-mass spectrometry (GC-MS) to evaluate the effect of *Radix phytolaccae* in rats. The rats were divided into three groups, the control group, *Radix phytolaccae* (Low, 0.5 g/kg) treated group, *Radix phytolaccae* (High, 1.0 g/kg) treated group. Compared to the control group, L-proline, 9H-purine, myo-inositol, arachidonic acid increased and d-glucose, D-glucopyranoside decreased in liver of the Low group. Compared to the control group, d-glucose decreased in liver of the High group. Compared to the control group, the glycine, myo-inositol increased in kidney of the Low group. Compared to the control group, urea and d-mannose increased; L-valine, L-leucine, mannose, L-threonine, L-proline, L-phenylalanine, 9H-purine decreased in kidney of the High group. Compared to the control group, d-mannose and L-tyrosine increased in kidney of the High group. The changes of metabolites increased or decreased, indicating that the effect of *Radix phytolaccae* could induce energy metabolism, amino acid metabolism, urea cycle perturbations in rats.

Keywords: Metabolomics, GC-MS, alprazolam, liver, kidney, heart, PLS-DA

Introduction

Traditional Chinese medicine *Radix phytolaccae* is the dried root of *Phytolacca acinosa Roxb* and *Phytolacca americana L* [1, 2]. The main component of *Radix phytolaccae* is triterpenoid saponin and sterols compounds, it also contains jaligonic acid, phytolaccagenin and esculentic acid, etc [3, 4]. Modern pharmacological researchers have found that *Radix phytolaccae* has multiple efficacies of antibiosis, antivirus, antiinflammatory, antitumor, enhancing immunity, eliminating phlegm and relieving asthma, etc [3].

In clinical practice, acute poisoning of *Radix phytolaccae* has been reported, patients have different degree of sympathetic activation and gastrointestinal stimulation symptoms, usually manifesting irritability, hypodynamia, dizziness headache, nausea vomiting, blurred vision, hyperreflexia, trance and alalia. In severe cases, it can cause hypotension, convulsion, coma, mydriasis, shock, cardiac or cardiorespiratory arrest even death.

Recently, metabolomics is widely used in the life sciences, including safety evaluation, drug development, screening of toxicity markers, disease diagnosis, gene function, etc [5-11]. This paper uses rats as research objects, sets multi-dose experimental groups, applies GC-MS to detect the changes of micromolecules in tissue after *Radix phytolaccae*, uses the principal component analysis (PCA) method to analyze the differences of metabolites and studies the effect of *Radix phytolaccae* on metabolism in rats.

Methods

Radix phytolaccae decoction

These raw materials (*Radix phytolaccae*) were obtained from the Second Affiliated Hospital &

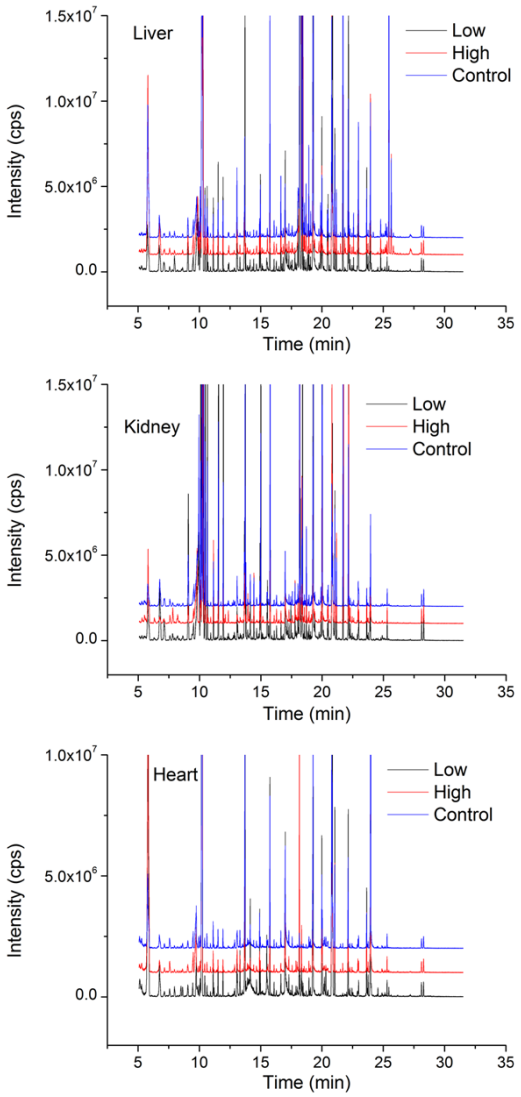

Figure 1. Typical GC-MS total ion chromatogram of rat liver, kidney and heart after *Radix phytolaccae*.

Yuying Children's Hospital of Wenzhou Medical University and stored in an environment of normal atmospheric pressure and decocted at 100°C for 30 minutes, and then the residues were discarded, the final decoction concentration was fixed at 1.0 g/mL. The decoction was stored at 4°C.

Metabolomics study

Thirty Sprague-Dawley rats (male, 220 ± 20 g) were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. The rats were divided into three groups, the control group, *Radix phytolaccae* (Low) treated group, *Radix phytolaccae* (High) treated group, 10 rats for each group. The control group was given normal saline by intragastric injection for 7 days. The *Radix phytolaccae* (Low and High) treated group, were given *Radix phytolaccae* decoction 0.5 and 1.0 g/kg one time by intragastric administration at every morning, and last for 7 days.

Tissue (liver, kidney and heart) samples were collected from the rats from three groups at 8:00 am after 8 day, respectively. The tissues were stored at -80°C until measurement. The metabolomics GC-MS conditions and sample preparation were set according to our previous work [12].

Data analysis

The GC-MS data was exported into Microsoft Excel, with the peaks normalized to the total sum of spectrum prior to multivariate analyses. The resulting data was processed through principal component analysis (PCA) and partial least squares discriminate analysis (PLS-DA) using SIMCA-P 11.5 software (Umetrics, Umea, Sweden).

Statistical analysis

Statistical analysis was carried out using SPSS software (Version 18.0, SPSS). Independent samples T-test was applied in order to detect significant differences in all metabolites between two groups. $P < 0.05$ was considered statistically significant.

Results

Figure 1 provides the typical metabolic profiles of tissues acquired through GC-MS technique. The endogenous metabolites in the tissues were identified using the NIST 2005 mass spectrometry database.

In order to explore the metabolic profile changes of the effect of *Radix phytolaccae* in rats, we compared the tissue results of PCA of *Radix phytolaccae* treated group with the rats in the control group (**Figure 2A**, **2D**, **2G**), the corresponding load diagram was shown in **Figure 2B**, **2E**, **2H**, PCA-3D were shown in **Figure 2C**, **2F**, **2I**. The tissue results of PLS-DA of *Radix phytolaccae* treated group with the rats in the control group (**Figure 3A**, **3D**, **3G**), the corre-

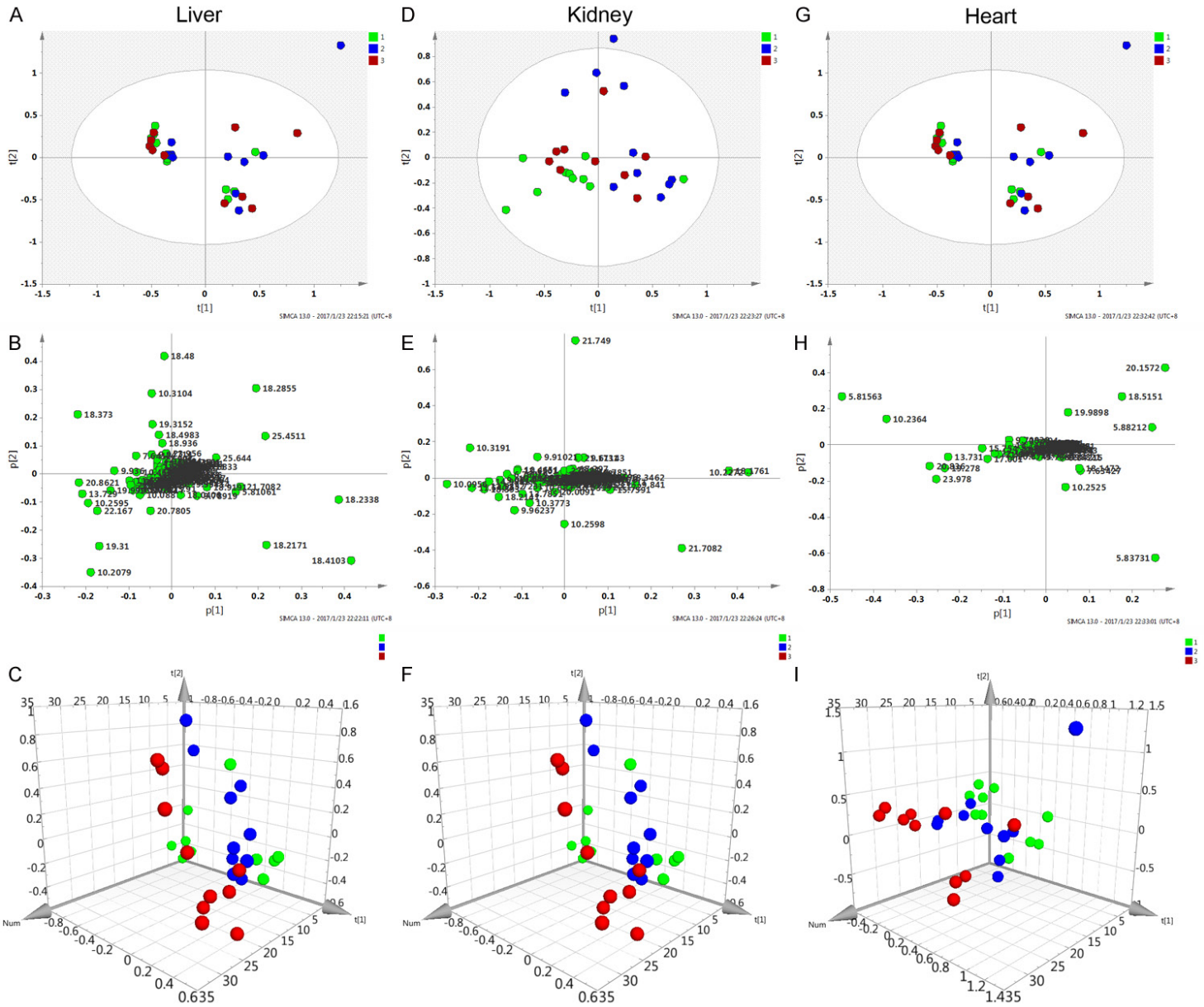

Figure 2. PCA score results of rat liver, kidney and heart samples (A, D, G) after *Radix phytolaccae*. Low (Class 1), High (Class 2), Control (Class 3); the corresponding load diagram (B, E, H), PCA-3D score results (C, F, I).

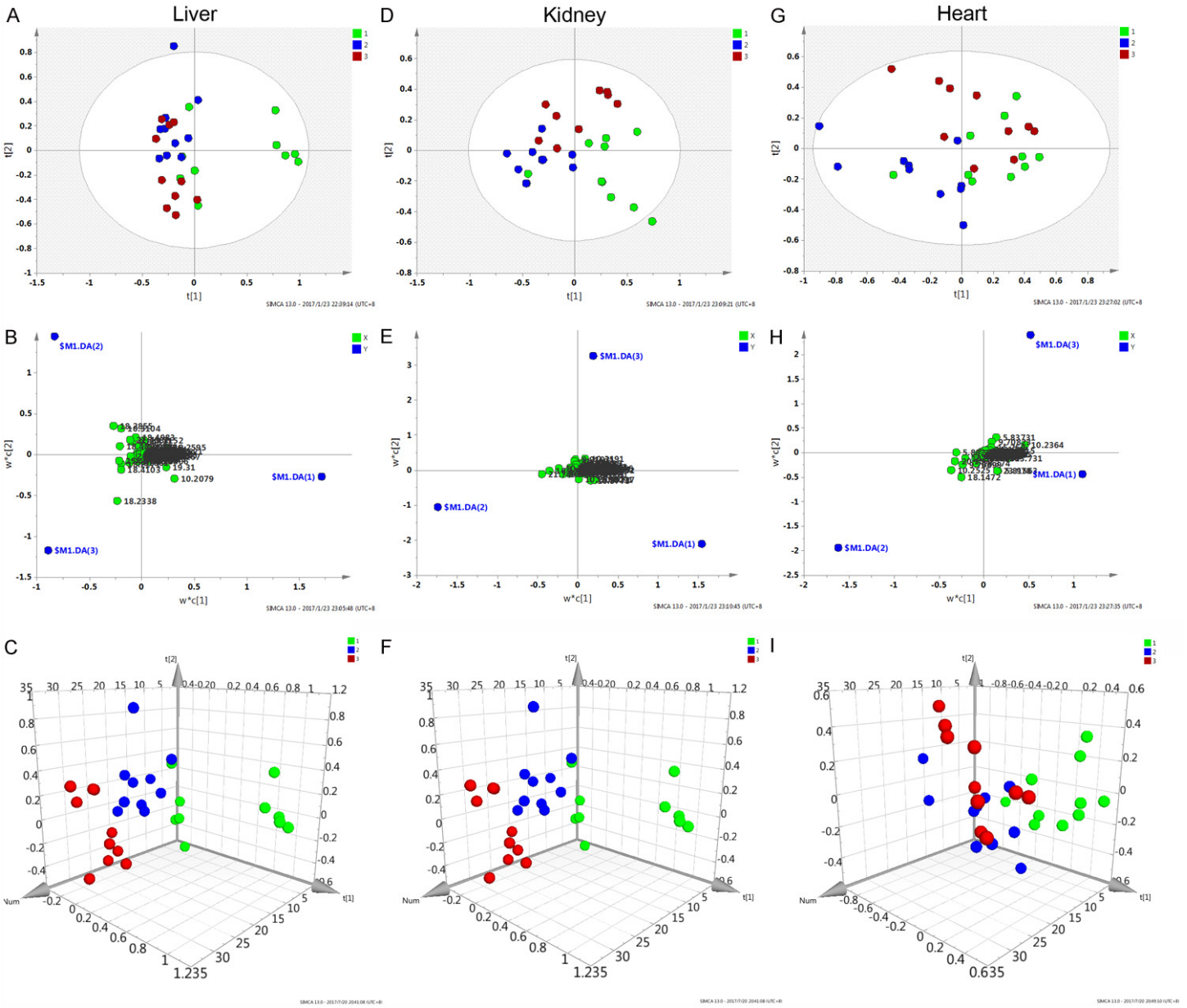

Figure 3. PLS-DA score results of rat liver, kidney and heart samples (A, D, G) after *Radix phytolaccae*. Low (Class 1), High (Class 2), Control (Class 3); the corresponding load diagram (B, E, H), PLS-3D score results (C, F, I).

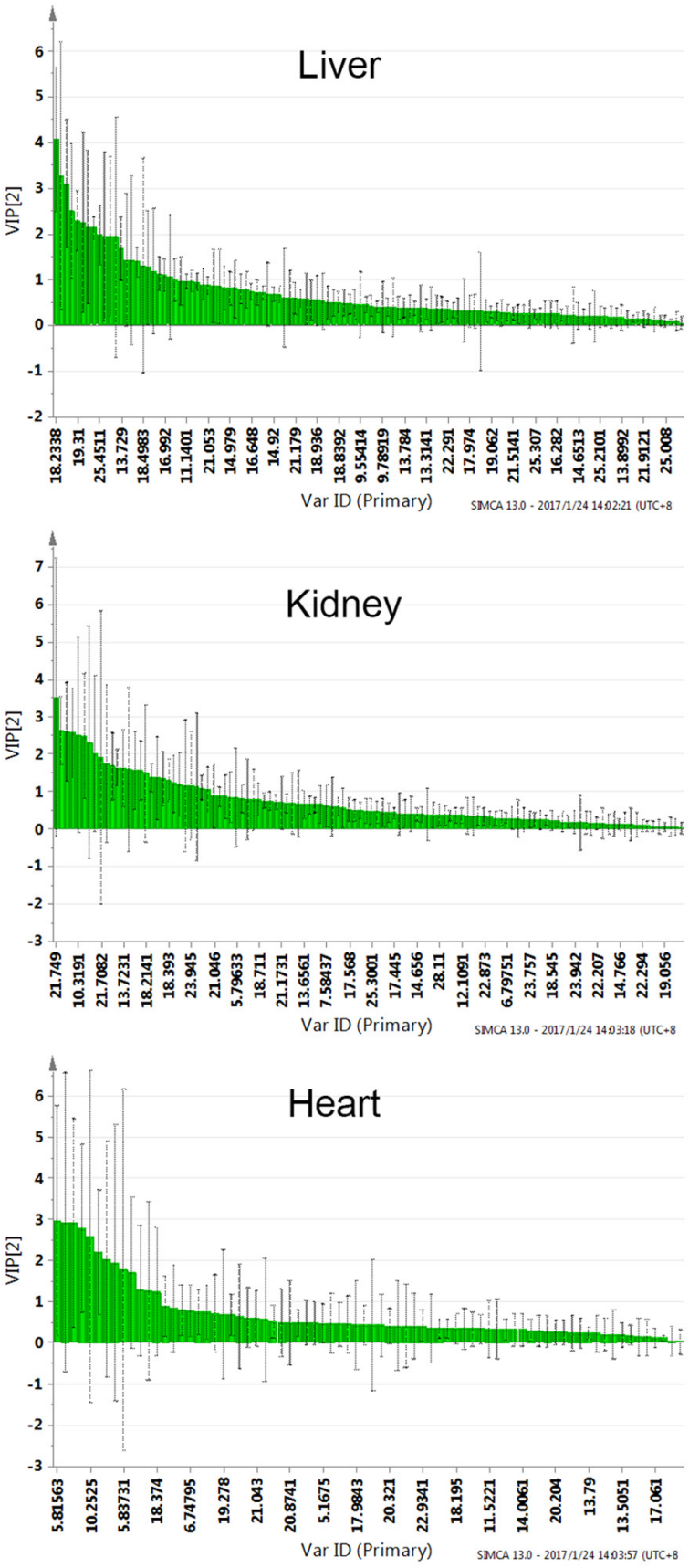

Figure 4. VIP was acquired from the PLS-DA model.

sponding load diagram was shown in **Figure 3B**, **3E**, **3H**, PLS-3D were shown in **Figure 3C**, **3F**, **3I**. Variable importance in the projection (VIP) was acquired from the PLS-DA model, **Figure 4**.

In this study, the changes of metabolites (VIP > 1) between *Radix phytolaccae* treated group and control group were shown in **Tables 1**-**3**. VIP was acquired from the PLS-DA model with a threshold of 1.0.

Discussion

Metabolomics is a newly emerging omics approach to the investigation of metabolic phenotype changes induced by environmental or endogenous factors [13-19]. PCA and PLS-DA were used to reveal the differences in tissue composition of two different groups, the corresponding loading plots, where each point represents a metabolite, were used to identify which variables contributed to the separation of the samples on the scores plot. In **Figures 2** and **3**, the results of the PLS-DA were better than PCA, and then we subsequently used a multivariate PLS-DA classification method to maximize metabolite variations and to identify the metabolites responsible for such variations, **Tables 1**-**3**.

Compared to the control group, L-proline, 9H-purine, myo-inositol, arachidonic acid increased and d-glucose, D-glucopyranoside decreased in

Table 1. Relative levels of metabolites in rat liver after *Radix phytolaccae*

NO.	Renten time/min	Metabolite	VIP	Low	High	Control
1	5.81	Butanoic acid	1.26	2.55	3.24	3.76
2	9.84	Urea	1.17	0.25	0.93	0.52
3	10.21	Glycerol	3.26	11.66	6.28	8.29
4	13.73	L-Proline	1.67	1.95*	0.84	0.73
5	16.99	9H-Purine	1.08	0.87**	0.48	0.47
6	18.23	d-Glucose	4.05	5.12*	6.13*	10.88
7	19.31	Hexadecanoic acid	2.28	6.21	3.78	4.48
8	20.00	Myo-Inositol	1.38	1.19**	0.55	0.51
9	22.17	Arachidonic acid	2.13	2.36**	0.95	0.96
10	25.45	D-Glucopyranoside	1.97	1.78*	3.13	3.47

Note: VIP was acquired from the PLS-DA model with a threshold of 1.0. Compared with control group, $*P < 0.05$ and $**P < 0.01$.

Table 2. Relative levels of metabolites in rat kidney after *Radix phytolaccae*

NO.	Renten time/min	Metabolite	VIP	Low	High	Control
1	9.08	L-Valine	1.10	1.29	0.68**	1.20
2	9.84	Urea	1.67	1.25	2.54*	1.25
3	9.96	Glycine	2.59	3.18*	0.89	0.86
4	10.10	Leucine	1.56	2.97	1.32	2.49
5	10.23	Glycerol	1.73	8.60	11.85	10.39
6	10.47	L-Leucine	1.35	2.22	1.65*	2.60
7	11.54	Mannose	1.63	2.34	0.96**	1.79
8	11.94	L-threonine	1.37	1.76	0.78**	1.44
9	13.72	L-Proline	1.62	3.47	1.84*	2.89
10	14.99	L-phenylalanine	1.33	1.94	1.16*	1.97
11	15.76	Fructose	1.13	3.58	4.27	3.42
12	16.99	9H-Purine	1.20	1.05	0.86**	1.29
13	18.18	d-Mannose	2.61	6.33	10.44*	7.74
14	19.28	Hexadecanoic acid	1.56	5.13	5.42	6.06
15	20.01	Myo-Inositol	2.55	8.73**	6.82	6.70
16	23.95	Mannonic acid	1.14	0.37	0.48	0.81

Note: VIP was acquired from the PLS-DA model with a threshold of 1.0. Compared with control group, $*P < 0.05$ and $**P < 0.01$.

Table 3. Relative levels of metabolites in rat heart after Radix phytolaccae

NO.	Renten time/min	Metabolite	VIP	Low	High	Control
1	5.82	Propanoic acid	2.96	8.66	5.71	5.69
2	10.24	Glycerol	2.77	20.91	15.26	20.27
3	13.73	L-Proline	2.20	5.88	3.64	4.23
4	18.15	d-Mannose	2.92	2.79	4.31*	1.26
5	18.37	L-Tyrosine	1.24	0.49	0.65*	0.17
6	23.98	Mannonic acid	2.91	10.63	8.51	7.91

Note: VIP was acquired from the PLS-DA model with a threshold of 1.0. Compared with control group, $*P < 0.05$.

liver of the Low group. Compared to the control group, d-glucose decreased in liver of the High group, **Table 1**. Compared to the control group, the glycine, myo-inositol increased in kidney of the Low group. Compared to the control group, urea and d-mannose increased; L-valine, L-leucine, mannose, L-threonine, L-proline, L-phenylalanine, 9H-purine decreased in kidney of the High group, **Table 2**. Compared to the control group, d-mannose and L-tyrosine increased in kidney of the High group, **Table 3**.

We demonstrated that tissue metabolomics methods by GC-MS could provide a useful tool to evaluate the effect of *Radix phytolaccae* in rats. The changes of metabolites increased or decreased, indicating that the effect of *Radix phytolaccae* could induce energy metabolism, amino acid metabolism, and urea cycle perturbations in rats.

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Disclosure of conflict of interest

None.

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